

A single adulticide dose of albendazole induces cytochromes P4501A in mouflon (*Ovis musimon*) with dicrocoeliosis

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ABSTRACT: Contact handling with wild or semi-domesticated animals requires limiting animal stress to minimum. In this respect, single administration of drug should be preferred in contact therapy of mouflon (*Ovis musimon*) infected by lancet fluke (*Dicrocoelium dendriticum*). We tested single administration of albendazole (ABZ) (30 mg/kg of body weight) in a form of oral suspension and investigated to reach anthelmintic effects and to modulate biotransformation enzymes in liver and small intestine. Two weeks after ABZ administration coprology and necropsy findings document the adulticide effect in liver. The activities of eight biotransformation enzymes and ABZ biotransformation were tested in hepatic and intestinal subcellular fractions from control and ABZ treated animals. The highest inductive effect of ABZ was detected on cytochromes P4501A (CYP1A) activities. Increased amount of CYP1A proteins was confirmed using western blotting. In hepatic and intestinal microsomes, velocity of albendazole sulfoxide (ABZSO) formation was unaffected, but a shift in ratio of individual ABZSO enantiomers was observed. The second step of ABZ biotransformation corresponding to the formation of the pharmacologically inactive albendazole sulfone, was significantly accelerated both in liver and intestine of ABZ treated animals. The increase of ABZ deactivation could facilitate the development of anthelmintic resistance in parasites. Although single ABZ dose is therapeutically effective, its potential to induce CYP1A should be taken in account for controlling helminthoses.

Keywords: biotransformation; benzimidazoles; lancet fluke; anthelmintic resistance

Dicrocoeliosis belongs in sheep and goats among the six economically most important pasture helminthoses (Hiepe, 1994). Spectrum of definitive hosts is very wide, includes ruminants, non-ruminants, and occasionally humans. Anthelmintic control of animal dicrocoeliosis is in respect to location of fluke adults in definitive hosts (thin bile ducts) difficult, several benzimidazoles (albendazole, fenbedazole, luxabendazole, mebendazole, thia-bendazole, cambendazole) or probenzimidazoles (netobimin, thiofanat), praziquantel, closantel, oxclosanid and diamphenetid were verified. Part of free living animal species (cervids, wild boar, etc.)

including mouflon (*Ovis musimon*) is at present considered in European conditions as game and farm species. It is generally accepted, that parasitically positive herds of mentioned species are treatable by administration of anthelmintics mixed with feed without any direct contact with humans. In such conditions, anthelmintics are dosed per kg of body weight in lower doses but administered in longer time schemes (Cordero Del Campillo et al., 1982; Dzakula et al., 1984; Kassai and Fok, 1985). An important exception is immobilized infected animal which is prepared for future breeding in new localities. In such cases, we can use contact

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treatment of animal. In mouflon dicrocoeliosis, selected anthelmintics can be administered via oral suspension. The similar experience with treatment of dicrocoeliosis in domestic sheep by single drug administration is satisfactory. Higher doses of anthelmintic, in comparison to treatment without direct contact, have to be used (Himonas and Liakos, 1980; Schuster and Hiepe, 1993). For handling reasons, single drug administration especially in game species is required simultaneously with wide anthelmintic efficiency (against adults, larvae, etc.) and helminthocide effect.

Biotransformation enzymes affect most of desired as well as undesired effects of drugs. Induction or inhibition of these enzymes can have significant pharmacological and toxicological consequences (Nebbia, 2001). If host biotransformation enzyme activities increased in course of antiparasitic administration, decrease of plasmatic levels of antiparasitics may lead to failure of therapy. The mentioned decrease in plasma levels of antiparasitics repetitively administered to the same animal population increases also the parasite chances to survive. It is well known that the only contact of parasites with low doses of antiparasitics results in the initiation of defence mechanisms of the parasite and subsequent lowering of sensitivity of the parasites to the used drugs (Geerts and Gryseels, 2000). The induction of biotransformation enzymes of host can thus indirectly contribute to development of the parasite's drug resistance.

In a previous study, we described that repeated albendazole (ABZ) administration to mouflon caused induction of cytochromes P4501A (CYP1A), which are responsible for ABZ deactivation (Velik et al., 2005). The present study was designed to evaluate the effect of a single ABZ administration to dicrocoelious mouflons in terms of both anthelmintic efficacy, and modulation of biotransformation enzymes activities involved in ABZ metabolism.

MATERIAL AND METHODS

Animals and biological material

Adult female mouflons selected from game park Vlkov (Czech Republic) were divided into three groups. Animals of the first group ($n = 5$) were individually treated with single dose of ABZ (30 mg/kg of body weight) by oral route, live body weight was recorded previously. ABZ suspension (10%) was

prepared in our laboratory. Vermitan (20% granulate, CEVA Phylaxia, Hungary) was powdered and dispersed into aqueous gel based on microcrystalline cellulose (Avicel RC-591, FMC, Belgium). Animals of the second group ($n = 5$) were used as untreated controls, suspension without ABZ was administered. Animals of these two experimental groups were culled 24 h after the administration of suspensions. Whole liver and 1.5 m of small intestine (measured from abomasum) were removed immediately and stored in liquid nitrogen during transport to laboratory. Third group of parasitologically positive animals (verified previously, $n = 4$) was used for experimental treatment, too. This group of mouflons was bred in small game enclosure, repeatedly caught and individually withdrawn for rectal samples of faeces. Animals were culled on 15th day after ABZ administration.

Helminthological control

Individual fecal samples of animals in third group were examined by use of modified McMaster method (Gringoli et al., 2004), number of fluke eggs per 1 g of sample (EPG) was determined. Liver of culled animals were sliced, repeatedly washed by physiological solution and fluke adults with eggs identified in sediment. Similar procedure was used for examination of gall bladder and terminal bile ducts contents.

Preparation of subcellular fractions

Frozen liver or small intestine were thawed at laboratory temperature (up to 15 min). Intestinal pieces were washed, cut open and mucosa was scrapped. Liver pieces and mucosa were homogenised at the w/v ratio of 1:6 in 0.1M sodium phosphate buffer, pH 7.4, using a Potter-Elvehjem homogeniser and sonication with Sonopuls (Bandeline, Germany). The microsomal fractions were isolated by fractional ultracentrifugation of the tissue homogenate with the same buffer. A re-washing step (followed by a second ultracentrifugation) was included at the end of the microsomes preparation procedure. Microsomes were finally resuspended in a buffer containing 20% glycerol (v/v) and stored at -80°C . Protein concentrations were assayed using the bicinchoninic acid method according to Sigma protocol.

Enzyme assays

Each enzyme assay was performed in triplicate for each animal. The amount of organic solvents in the final reaction mixtures did not exceed 0.1% (v/v).

The activities of several isoforms of CYPs P450 were quantified. The 7-ethoxyresorufin (EROD), 7-methoxyresorufin (MROD), 7-pentoxoresorufin (PROD) *O*-dealkylases and 7-benzyloxyresorufin *O*-dearylase (BROD) activities were determined at 37°C using fluorimetric determination of resorufin (Weaver et al., 1994). Each substrate (dissolved in dimethylsulfoxide (DMSO)) was added at a final concentration of 5 µM. The amount of microsomal protein in the reaction mixture ranged between 0.15 to 0.20 mg. Assays were conducted using the Perkin-Elmer luminescence spectrophotometer LS 50B with the excitation and emission wavelength of 530 nm and 585 nm, respectively. The EROD, MROD, PROD, and BROD activities were calculated using the standard amount – addition technique.

The 7-methoxy-4-trifluoromethylcoumarin demethylase (MFCD) activity was measured using fluorimetric determination of 4-trifluoromethylumbelliferone. The final concentration of substrate (dissolved in DMSO) was 20 µM. The amount of microsomal protein in the reaction mixture ranged between 0.2–0.3 mg. The excitation and emission wavelengths of 410 nm and 510 nm were respectively used (Crespi and Stresser, 2000).

6β-testosterone hydroxylase activity (6β-TOH) was assayed using the method described by Reinerinck et al. (1991) with slight modifications (Skalova et al., 2001). The final concentration of substrate (dissolved in methanol) was 250 µM and the amount of microsomal protein was 0.6–0.8 mg. The amount of 6β-hydroxytestosterone was determined by HPLC.

The activity of flavine monooxygenases toward thiobenzamide (TBSO) was assayed using the method of Cashman and Hanzlik (1981). The final concentration of substrate (dissolved in acetonitrile) was 1 mM. The formation of the metabolic product (*S*-oxide of thiobenzamide) was followed using the spectrophotometer at 370 nm.

The microsomal UDP-glucuronosyl transferases (UGT) activity was assayed following the method described by Mizuma et al. (1982). The reaction mixture (total volume 0.1 ml) contained 10 µl of microsomes (60–80 µg of protein), 3.3 µM UDP-glucuronic acid, 3.3 µM *p*-nitrophenol (dissolved in

redistilled water) in 0.1M Tris/HCl buffer, pH 7.4. After 20 min of incubation at 37°C, the reaction was stopped by addition of 50 µl of 3% trichloroacetic acid. After shaking and centrifugation, 50 µl of the supernatant was mixed with 50 µl of 1M NaOH and absorbance was measured by microplate reader BioRad (detection wavelength 415 nm).

Western blotting

The procedure used for electrophoretic separation and transfer of microsomal protein was described previously (Soucek et al., 1995; Skalova et al., 2001). The amount of proteins per line was 20 µg. Blots were incubated one hour at 37°C with a primary antibody. Polyclonal goat IgG against rat CYP1A1/2 (Daiichi Chemicals, Tokyo, Japan) were used, diluted 1:1 000 with buffer containing 20mM potassium phosphate (pH 7.4) with 150mM NaCl, 0.05% Tween 20 (w/v) (PBST) and 0.5% blotting milk. After extensive washing, incubation for 30 min at laboratory temperature with rabbit anti-goat IgG conjugated with alkaline phosphatase (Pierce, Illinois, USA), dilution 1:10 000, followed. Blots were washed in 0.1M Tris buffer pH 9.5, covered with chemiluminescent substrate DuoLux (Vector Lab, Burlingame, USA) and incubated for 5 min. The membranes were then exposed to X-ray film Medix XBU (Foma, Hradec Kralove, Czech Republic). Films were developed (standard developing process) and image was recorded with a scanner (Hewlett-Packard Scanjet 3570c). Densitometry was used for quantification (LabImage 1D gel analysis software, Kapelan GmbH, Halle, Germany).

Incubation of microsomes with ABZ

The hepatic or intestinal microsomal fractions were incubated with ABZ (20 µM). The reaction mixture (total volume of 0.3 ml) contained 50 µl of microsomal suspension containing 0.4–0.6 mg of proteins, NADPH (0.1mM) and 0.1M Na-phosphate buffer, pH 7.4. The blank samples contained 50 µl of 0.1M phosphate buffer, pH 7.4, instead of microsomes. The incubations were carried out at 37°C for 30 min under aerobic conditions. At the end of incubation, 180 µl of cooled acetonitrile were added, shaken (3 min, vortex) and centrifuged (10 min, 10 000 g). Supernatants were analysed using HPLC.

HPLC analysis

Achiral HPLC was carried out using a Shimadzu LC-10ADvp solvent delivery module, a Shimadzu SIL-10ADvp autoinjector, a Shimadzu RF-10Axl fluorescence detector ($\lambda_{EX} = 290$ nm, $\lambda_{EM} = 320$ nm), a Shimadzu CTO-10ACvp column oven, fitted with a LiChroCART 250-3 (LiChrospher 60 RP-select B, 250 mm \times 3 mm, 5 μ m) reverse-phase HPLC column (Hewlett Packard, USA) equipped with LiChroCART 4-4 (LiChrospher 60 RP-select B, 4 mm \times 4 mm, 5 μ m) guard column (Merck, Germany). Mobile phase A consisted of acetonitrile/25mM potassium phosphate buffer (pH 3.0) (35:65, v/v) with a flow rate of 0.5 ml/min in isocratic mode. All experiments were carried out at 25°C. Data were processed using the Shimadzu Class VP integrator software, version 6.12 SP2. The compounds were identified in respect of the retention times of reference standards (Schering Plough, NY, USA; purity 99%). The standards were kind gifts of C. Lanusse (Argentina). Under these chromatographic conditions, the retention times were 5.1 min (total-ABZSO), 7.1 min (ABZSO₂), and 17.2 min (ABZ). The limit of detection for ABZ, ABZSO and ABZSO₂ was 50.0, 20.0, and 2.0 ng/ml, respectively. The linear calibration curves in range 4–16 μ g/ml (ABZ), 0.5 to 3 μ g/ml (ABZSO) and 0.027–0.162 μ g per ml (ABZSO₂) served for quantification.

During the reverse phase HPLC analysis, the total-ABZSO chromatographic peak fractions were collected into vials. The collected fractions were evaporated to dryness using Eppendorf 5310 concentrator and redissolved in 250 μ l of mobile

phase B (0.5% 2-propanol in 0.01M phosphate buffer solution, pH 6.9).

Hundred μ l of each sample were injected on to a Shimadzu HPLC system fitted with a chiral Chiral-AGP stationary phase (150 mm \times 4 mm, 5 μ m) column (ChromTech, Hägersten, Sweden) equipped with Chiral-AGP (10 mm \times mm, 5 μ m) guard column. The flow rate of the mobile phase B was 0.9 ml/min. This chiral chromatographic method was adapted from that described previously by Delatour et al., 1990. ABZSO enantiomers were identified after chromatographic analysis of the racemic standard of this molecule. The retention time was 6.7 min for (–)-ABZSO and 15.4 min for (+)-ABZSO.

Statistical analysis

Experimental data are presented as a mean \pm standard deviation. One-way ANOVA was used for statistical evaluation of differences between ABZ treated and control animals.

RESULTS

Parasitological findings

Pre-treatment EPG values found in mouflons of the third experimental group were very high (EPG = 1023 \pm 218). The post-treatment EPG values were derived in % of mean pre-treatment findings the course of these values is presented in Figure 1. It is

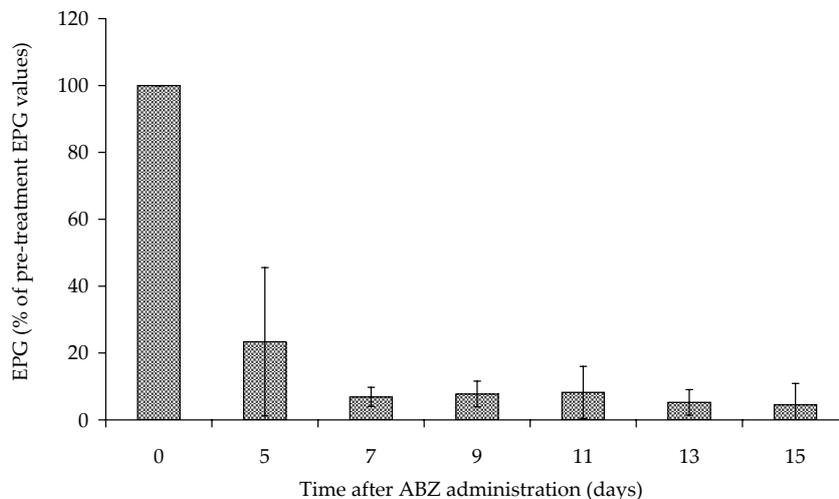


Figure 1. The amount of *Dicrocoelium dendriticum* eggs excreted per 1g of faeces (EPG) after albendazole administration in mouflons

Table 1. Specific activities of 7-ethoxyresorufin (EROD), 7-methoxyresorufin (MROD), 7-penthoxyresorufin (PROD) *O*-dealkylase and 7-benzyloxyresorufin *O*-dearylase (BROD) tested in hepatic and intestinal microsomes of control and albendazole (ABZ) treated animals

Biological tissue	Experimental group	EROD	MROD	PROD	BROD
		(pmol/min/mg of proteins)			
Liver	control	54.8 ± 17.6	43.9 ± 18.9	1.0 ± 1.0	10.4 ± 3.4
	ABZ treated	50.0 ± 5.7	26.6 ± 3.6	2.5 ± 1.8	7.0 ± 2.9
Intestine	control	1.4 ± 1.2	0.09 ± 0.08	ND	ND
	ABZ treated	34.5 ± 17.3*	1.36 ± 1.03*	ND	0.96 ± 0.93*

Data represent the mean ± SD from 5 individual animals. Each sample was made in triplicate

ND = not detected

*statistically significant difference from control $P \leq 0.001$

evident, that ABZ administration was anthelmintically very effective, fecal EPG values are rapidly decreasing and by 7th day after the treatment stay very low. The control of individual livers and biles confirmed coprological findings, few dead fluke adults and low content of fluke eggs in sediments were found only.

Effect of ABZ treatment on CYP1A

In hepatic and intestinal microsomes of control and ABZ treated animals the activities of CYP1A enzymes (EROD, MROD) were measured. The amount of protein corresponding to CYP1A was determined immunochemically by western blotting and quantified using densitometry (Table 1 and Figure 2). Intestinal microsomes from ABZ treated animals exhibited approximately 25-fold higher EROD activity than control microsomes. ABZ treatment of mouflons led also to significant

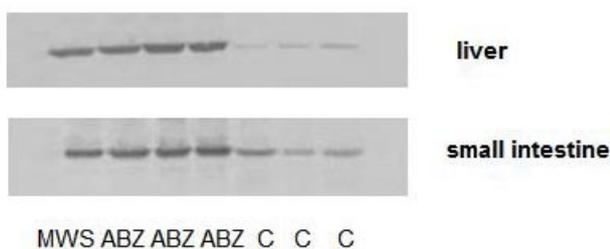


Figure 2. Immunoblotting analyses of samples of hepatic and intestinal microsomes of control (C) or albendazole (ABZ) treated mouflons with CYP1A1/2 antibodies. MWS = molecular weight standard (the line corresponding to 50kD)

($P \leq 0.001$) increase of MROD activity (approximately 15-fold). Intestinal CYP1A protein was significantly enhanced in ABZ treated animals compared to controls. Also in liver, ABZ treatment led to significant increase of CYP1A corresponding protein. On the other hand, no changes in hepatic EROD or MROD activities caused by ABZ treatment were detected.

Effect of ABZ treatment on activity of other biotransformation enzymes

Activities of PROD, BROD, MFCD, 6 β -TOH, TBSO and pn-UGT were measured in hepatic and intestinal microsomes (Tables 1 and 2). Intestinal microsomes did not exhibit any PROD, BROD, MFCD and TBSO activity. Intestinal 6 β -TOH activity was very low, approximately 100 times less extensive than in hepatic microsomes. ABZ treatment of mouflons led to significant enhancement of intestinal BROD activity (none BROD activity was detected in controls). In hepatic microsomes, ABZ treatment caused significant inhibition of MFCD activity. No other differences were observed in tested activities between control and ABZ-treated animals.

In vitro metabolism of ABZ in microsomes of control and ABZ treated mouflons

Before incubations, presence of ABZ or its metabolites in microsomal suspension from ABZ treated animals were assayed. In hepatic microsomes, low amount of ABZ and both metabolites

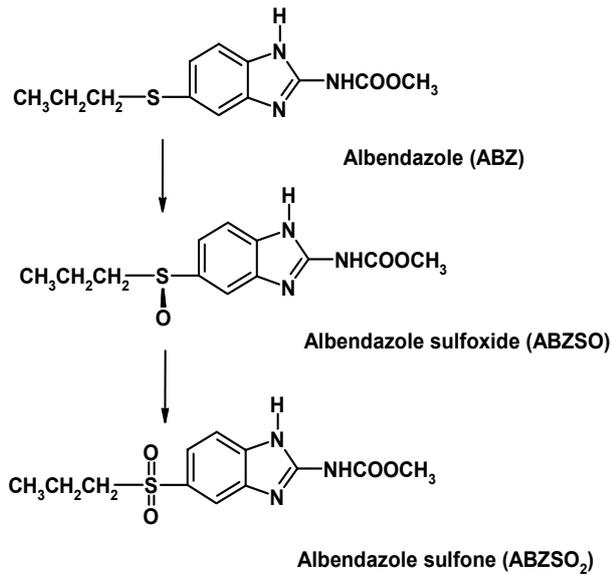


Figure 3. The structure of ABZ and its main metabolites

were found. In intestinal microsomes, none ABZ, low amount of ABZSO and only traces of ABZSO₂ were detected (Table 3). With aim to compare the velocity of ABZ oxidation, ABZ at saturated concentration was incubated with NADPH and either hepatic and intestinal microsomes of control and ABZ treated mouflons. Concentration of ABZ, ABZSO and ABZSO₂ was measured in reaction mixture using HPLC (Figures 3 and 4). In blank samples (without microsomes) none metabolite of ABZ was detected. ABZ treatment did not sig-

nificantly affect the velocity of formation of total ABZSO in hepatic microsomes. Mild increase of ABZSO formation was observed in intestinal microsomes from ABZ treated animals compared to controls. As ABZSO exists in two enantiomeric forms chiral stationary phase was used for separation of ABZSO enantiomers. In liver, ABZ oxidation is stereospecific with preferential formation of (+)-ABZSO (ratio 2.6). In small intestine, the formation of (-)-ABZSO stood over the formation of (+)-ABZSO, but the differences between both enantiomers was only mild (ratio 1.3). Effect of ABZ treatment on stereospecificity of *in vitro* ABZSO formation is shown in Figure 5. While ABZ treatment did not affected ratio of ABZSO enantiomers formed in small intestine, in hepatic microsomes ABZ treatment caused significant ($P \leq 0.001$) shift of stereospecificity toward (-)-ABZSO.

To study the second step of ABZ sulfoxidation, the formation of ABZSO₂ in microsomes from control and ABZ treated animals was compared. Figure 6 demonstrates the significant differences in velocity of ABZSO₂ formation. ABZ administration caused approximately 6.8 fold and 4.4 fold increases in velocity of ABZSO₂ formation in microsomes from liver and small intestine, respectively.

DISCUSSION

The microcoelious animals used for breeding in new or parasitically negative localities can serve

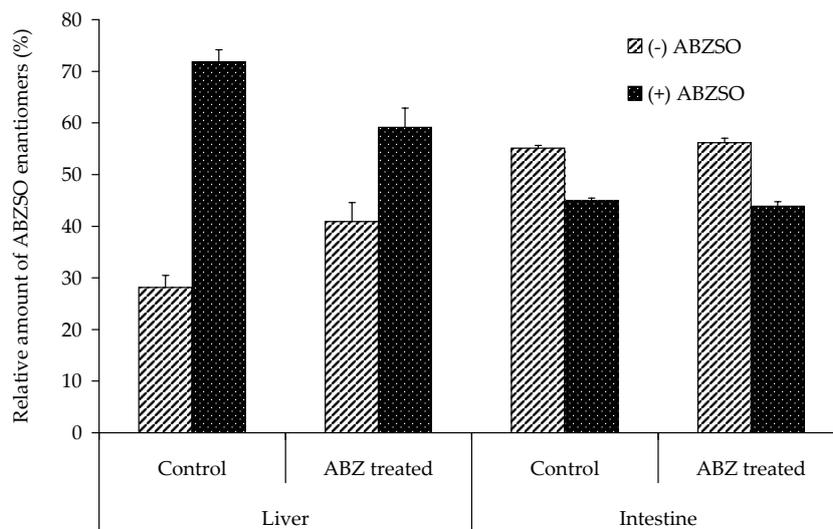


Figure 4. Stereospecificity of albendazole sulfoxide (ABZSO) formation in hepatic and intestinal microsomes of control and albendazole (ABZ) treated animals. Stereospecificity is expressed as percentage ratio of ABZSO enantiomers formed. Concentration of total ABZSO e.g. sum of (+)-ABZSO and (-)-ABZSO = 100%

Table 2. Specific activities of 6 β -testosterone hydroxylase (6 β -TOH), 7-methoxy-4-trifluoromethylcoumarin demethylase (MFCD), thiobenzamide S-oxidase (TBSO) and UDP-glucuronosyl transferase toward *p*-nitrophenol (np-UGT) tested in hepatic and intestinal microsomes of control and albendazole (ABZ) treated animals

Biological tissue	Experimental group	6 β -TOH	MFCD	TBSO	pn-UGT
		(nmol/min/mg of proteins)			
Liver	control	0.36 \pm 0.07	0.72 \pm 0.14	0.32 \pm 0.05	7.99 \pm 2.36
	ABZ treated	0.31 \pm 0.03	0.36 \pm 0.05*	0.20 \pm 0.17	6.66 \pm 0.73
Intestine	control	0.02 \pm 0.01	ND	ND	0.10 \pm 0.09
	ABZ treated	0.04 \pm 0.02	ND	ND	0.23 \pm 0.19

Data represent the mean \pm SD from 5 individual animals. Each sample was made in triplicate

ND = not detected

*statistically significant difference from control $P \leq 0.001$

Table 3. The amount of albendazole (ABZ) and its metabolites albendazole sulfoxide (ABZSO) and albendazole sulfone (ABZSO₂) detected in hepatic and intestinal microsomes of ABZ treated animals

Biological tissue	ABZ	ABZSO	ABZSO ₂
	(nmol/mg of protein)		
Liver	0.97	0.42	0.170
Intestine	ND	0.24	0.003

Data represent the mean of two HPLC analyses of microsomes mixture from 5 individual animals

ND = not detected

there as a source of infection for microcoeliosis free animals and simultaneously the infection is burden for themselves for the rest of their live (Kirkwood and Peirce, 1971). To protect this unwanted situation it is necessary to find effective control approach limiting in minimum possibility of spreading of fluke infection. From this point of view, data reached in our study are favourable. Rapid decrease in EPG coprological values, recovery of dead flukes in liver and minimal content of fluke eggs in bile, document that single ABZ administration is able to break off definitively the infection caused by *Dicrocoelium dendriticum* in mouflon. In time decreasing parasitological findings documented in our paper are consequences of rewashing of bile ducts in liver, which some of them are anatomically severely destroyed. Due to this change completely zero parasitological findings can be expected later on. Based on presented data the effect of ABZ used in our study can be described as an adulticide.

With respect to risk of drug resistance of helminths, it is very important to study not only antiparasitic efficacy of ABZ but also the modulation of biotrans-

formation enzymes, since the development of the drug resistance can be facilitated by the induction of host liver biotransformation enzymes. ABZ is a well-known inducer of biotransformation enzymes, mainly CYP1A subfamily in rats (El Amri et al., 1988; Asteinza et al., 2000; Baliharova et al., 2003a), in human, rabbit and HepG2 cells (Rolin et al., 1989; Galtier, 1991; Galtier et al., 1997; Baliharova et al., 2003b). However, extrapolation of the data obtained in rodents and human to game or farm animals is not possible because of interspecies differences in regulation of CYPs. Hence, induction studies in target species such as mouflon were initiated. This study proved strong induction of CYP1A in mouflon liver and small intestine after repeated ABZ administration (Velik et al., 2005). In the present study, the effects of a single dose administration of ABZ on biotransformation enzymes and ABZ metabolism was tested.

The administration of single therapeutic ABZ dose to mouflons led to strong increase of EROD and MROD activity in intestinal microsomes, these activities are ascribed to CYP1A1/2 (Weaver et al.,

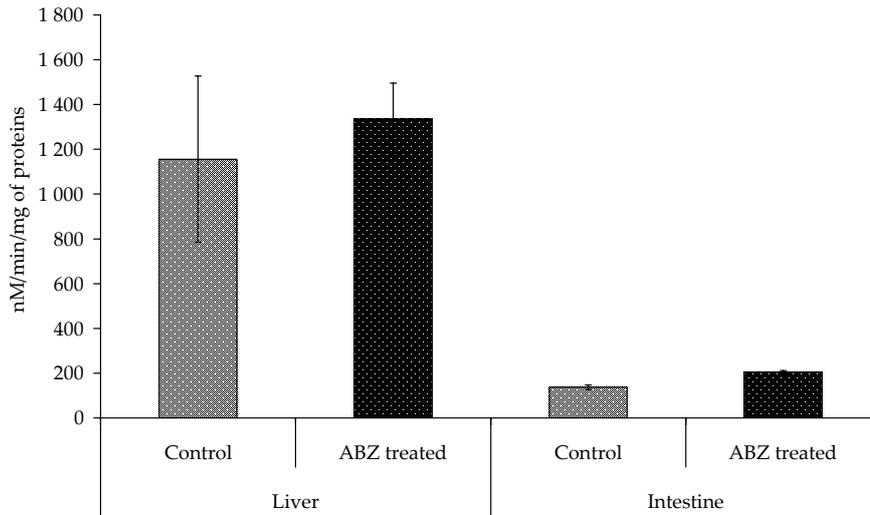


Figure 5. The specific velocity of albendazole sulfoxide (ABZSO) formation in hepatic and intestinal microsomes of control and ABZ treated animals. Concentration of albendazole substrate was 20 μ M in reaction mixture

1994). The increase of amount of intestinal CYP1A proteins was confirmed by immunodetection. In liver, ABZ caused the significant increase of CYP1A protein, but none increase of CYP1A activities. This disagreement may be explained by inhibition of CYP1A activities by ABZ and ABZSO residues in microsomes isolated from ABZ treated animals, because inhibitory effects of ABZ and ABZSO on EROD activity in mouflon microsomes was documented (Baliharova et al., 2005). Besides CYP1A, single ABZ dose caused significant increase of BROD activity (corresponding to CYP3A and CYP2B) (Weaver et al., 1994) in intestinal microsomes and significant

inhibition of hepatic MFC activity (corresponding mainly to CYP2C) (Crespi and Stresser, 2000). Modulation of these enzyme activities could alter own ABZ metabolism and metabolism of simultaneously or consecutively administered drugs that are metabolised by these enzymes.

The influence of ABZ administration to mouflons on metabolism of ABZ itself was tested *in vitro* in hepatic and intestinal microsomes. In liver as well as in small intestine, ABZ is metabolised through two-step *S*-oxidation giving firstly chiral albendazole sulfoxides (ABZSO) followed by albendazole sulfone (ABZSO₂).

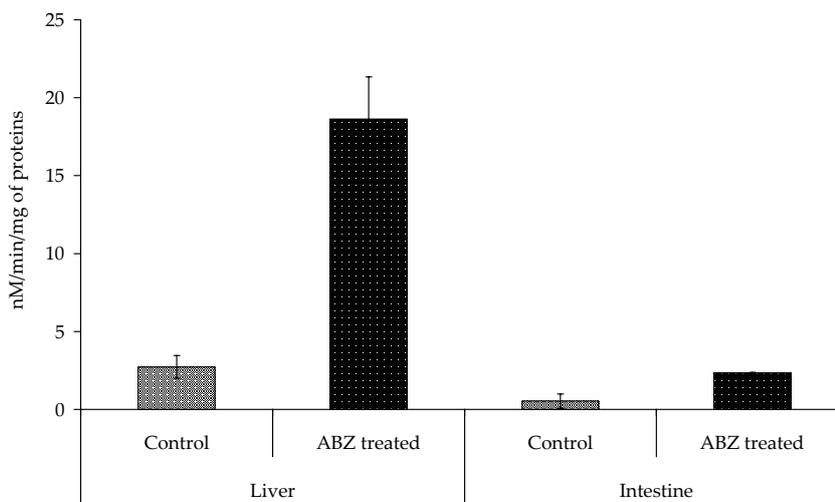


Figure 6. The specific velocity of albendazole sulfone (ABZSO₂) formation in hepatic and intestinal microsomes of control and ABZ treated animals. Concentration of substrate (ABZ) was 20 μ M in reaction mixture

The formation of pharmacologically active ABZSO is mainly ascribed to CYP3A and FMO (Fargetton et al., 1986; El Amri et al., 1987; Delatour et al., 1991; Moroni et al., 1995). ABZ administration did not affect the ABZSO formation in hepatic microsomes. These results are in agreement with testing of ABZ effect on biotransformation enzyme activities such as hepatic 6 β -TOH, BROD and TBSO (reflecting CYP3A and FMO activities) (Reinerink et al., 1991, resp. Cashman and Hanzlik, 1981) were not affected, too. On the other hand, a significant increase in ABZSO formation in intestinal microsomes was observed in ABZ treated animals. This result may correspond to increasing intestinal BROD activity, but participation of other ABZ-inducible enzyme (e.g. CYP1A) on ABZSO formation in mouflon small intestine can not be excluded.

The formation of pharmacologically inactive ABZSO₂ (second step of ABZ sulfoxidation) is mainly catalysed by CYP1A (El Amri et al., 1988). A significant increase of ABZSO₂ formation was observed in hepatic as well as in intestinal microsomes from ABZ treated animals. This finding proved that treatment of mouflons with single ABZ dose significantly induced CYP1A1/2 in liver and small intestine and led to acceleration of ABZ deactivation.

Described acceleration in deactivation of ABZ following single higher dose of anthelmintic is problematic as well as repeated administration of lower ABZ doses. Thus in therapy of mouflon and probably also in related domestic sheep, such consequences of ABZ administration should be taken into account whereas the use of another suitable anthelmintic without inductive effect should be initiated.

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